CLAIMS

What is claimed is:

1. A method of treating vesicles with exogenous material for insertion of the exogenous material into the vesicles, comprising the steps of:

a. retaining a suspension of the vesicles and the exogenous material in a treatment volume in a chamber which includes electrodes, wherein the chamber has a geometric factor (cm^{-1}) defined by the quotient of the electrode gap squared (cm^2) divided by the chamber volume (cm^3),

wherein said geometric factor is less than or equal to 0.1 cm^{-1}),

wherein the suspension of the vesicles and the exogenous material is in a medium which is adjusted, such that the medium has conductivity in a range spanning 0.01 to 1.0 milliSiemens,

wherein the suspension is enclosed in the chamber during treatment, and

b. treating the suspension enclosed in the chamber with one or more pulsed electric fields,

wherein in accordance with a. and b. above, the treatment volume of the suspension is scalable, and

wherein the time of treatment of the vesicles in the chamber is substantially uniform.

2. The method of claim 1 wherein the chamber is a closed chamber.

3. The method of claim 1 wherein the chamber has at least a 2 milliliter capacity.

4. The method of claim 1 wherein the chamber and the contents thereof are sterile.

5. The method of claim 1 wherein the chamber includes entry and exit ports for entry and removal of the suspension.

6. The method of claim 1 wherein the electrodes are parallel plate electrodes.

7. The method of claim 1 wherein the electric fields are substantially uniform throughout the treatment volume.

8. The method of claim 1 wherein the electric fields include a rectangular voltage pulse waveform to produce a uniform pulse electric field between parallel plate electrodes greater than 100 volts/cm and less than 5,000 volts/cm, substantially uniform throughout the treatment volume.

9. The method of claim 1 wherein:
the vesicles are living cells,
the medium is a physiological medium and has a conductivity between 50 and 500 μS/cm.

10. The method of claim 1 wherein the living cells being treated are at least 10 million in number.

11. The method of claim 1 wherein the living cells being treated are at least 20 million in number.

12. The method of claim 1 wherein the vesicles are autologous cells that are to be returned to a donor after treatment with the exogenous material.

13. The method of claim 1 wherein the vesicles are syngeneic cells that are to be given to a recipient other than the donor.

14. The method of claim 1 wherein the vesicles are

zenogeneic cells.

15. The method of claim 1 wherein the vesicles are artificial liposomes.

16. The method of claim 1 wherein the pulsed electric fields are from electrical pulses which are in a sequence of at least three non-sinusoidal electrical pulses, having field strengths equal to or greater than 100 V/cm, to the material, wherein the sequence of at least three non-sinusoidal electrical pulses has one, two, or three of the following characteristics: (1) at least two of the at least three pulses differ from each other in pulse amplitude; (2) at least two of the at least three pulses differ from each other in pulse width; and (3) a first pulse interval for a first set of two of the at least three pulses is different from a second pulse interval for a second set of two of the at least three pulses.

17. The method of claim 1 wherein temperature rise during treatment is miniscule.

18. The method of claim 1 which is scalable in a range spanning 2 to 10 milliliters.

19. The method of claim 1 which is carried out in sequential batches.

20. The method of claim 1 wherein the exogenous material is a therapeutic material.

21. The method of claim 1 wherein a therapeutic product is formed from the treatment of the vesicles with exogenous material.

22. The method of claim 1 wherein the exogenous material is a polynucleotide.

23. The method of claim 1 wherein the exogenous material is selected from the group consisting of DNA and RNA.

24. The method of claim 1 wherein the exogenous material is a polypeptide.

25. The method of claim 1 wherein the exogenous material is a protein.

26. The method of claim 1 wherein the exogenous material is an organic compound.

27. The method of claim 1 wherein the exogenous material includes at least eight base pairs.

28. The method of claim 1 wherein the chamber has a chamber volume, the suspension has a suspension volume, and the suspension volume is greater than the chamber volume, and wherein

an initial portion of the suspension volume is moved into the chamber, retained and treated in the chamber, and moved out from the chamber, and

an additional portion of the suspension volume is moved into the chamber, retained and treated in the chamber, and moved out from the chamber.

29. The method of claim 1 wherein still further portions of the suspension volume are sequentially moved into the chamber, retained and treated in the chamber, and moved out from the chamber.

30. The method of claim 1 wherein still further portions of the suspension volume are sequentially moved into the chamber, retained and treated in the chamber, and moved out from the chamber until the suspension volume is depleted.

31. An electroporation apparatus, comprising:

a chamber having a chamber volume of at least 2 milliliters, a pair of electroporation electrodes contained within said chamber,

an electroporation medium, carrying vesicles in suspension, contained in said chamber between said electroporation electrodes, wherein said medium has a conductivity between 50 and 500 mS/cm,

a source of pulsed voltages electrically connected to said electroporation electrodes, and

means for adding material to said chamber for electroporation treatment therein, and means for removing treated material from said chamber.

32. The apparatus of claim 31, further including sealing means connected to said chamber for providing a sealed chamber.

33. The apparatus of claim 31 wherein said sealing means include a quantity of elastomer material.

34. The apparatus of claim 31 wherein said sealed chamber is sterile inside the chamber.

35. The apparatus of claim 31 wherein said chamber includes vent means for venting air when fluid is moved into said chamber.

36. The apparatus of claim 31 wherein said vent means include a filter member in a wall of said chamber.

37. The apparatus of claim 31 wherein said vent means include a vent cell in fluid communication with said chamber.

38. The apparatus of claim 31 wherein said chamber includes a chamber inlet and a chamber outlet.

39. The apparatus of claim 31, further including:

a first reservoir, in fluid communication with said chamber inlet, for containing said vesicle-bearing electroporation medium prior to introduction into said chamber,

a second reservoir, in fluid communication with said chamber inlet, for containing a chamber flushing material for flushing treated vesicle-bearing medium out from said chamber, and

a third reservoir, in fluid communication with said chamber outlet, for receiving treated, vesicle-bearing medium that is flushed out from said chamber.

40. The apparatus of claim 39 wherein said first reservoir, said second reservoir, and said third reservoir are comprised of flexible bags.

41. The apparatus of claim 31 , further including:

an inlet valve connected between said chamber inlet and said first reservoir and said second reservoir, and

an outlet valve connected between said chamber outlet and said third reservoir.